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Attorney's Docket No.: 12875-002001 / 0643-5299US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Wei-Yu Lo et al.
Serial No. : 09/778,516
Filed : February 7, 2001
Title : LAC SHUTTLE VECTORS

Art Unit : 1632
Examiner : Pappu, Sita S

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF DR. WEI-YU LO UNDER 37 C.F.R. § 1.132

Considered
SSP
05/13/02

I, Wei-Yu Lo, Ph.D., hereby declare:

1. I am a co-inventor of the subject matter claimed in the above-referenced application, which relates to a novel Lac shuttle vector.

2. I, or others under my supervision, constructed an alpha-fetoprotein (AFP)-expression Lac shuttle vector, and successfully used it to generate immune response in mice.

More specifically, an AFP gene, a tumor-associated antigen gene, was inserted into pCLP7, a Lact shuttle vector (see Fig. 4 of this applicaiton), to form pCLP7/AFP. Both pCLP7 and pCLP7/AFP were introduced into *Lactobacillus casei* cells by transformation. After the transformed cells had grown in a selection medium for 24 hr, the cell numbers were adjusted to 1 x 10^{10} cfu/mL in phosphate-buffered saline (PBS). The cells were then heat-killed at 100°C for 5 min. A 100 μL pCLP7/AFP-transformed *L. casei* suspension or 100 ng naked AFP DNA (pcDNA/AFP) was administered intramuscularly (i.m.) to each mouse. Naked AFP DNA was used as a positive control, and pCLP7-transformed *L. casei* suspension was used as a negative control. Serum was collected from the tails of the mice biweekly.

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

April 22, 2002
Date of Deposit

Deborah R. Dast
Signature

DEBORAH R. DAST
Typed or Printed Name of Person Signing Certificate

Purified AFP, at a 10 μg/mL concentration, was coated onto a 96-well microtiter plate via overnight incubation at 4°C. The coated plate was washed twice with PBS containing 0.05% Tween-20, and incubated with 10% fetal bovine serum (FBS) in PBS for 2 hr at room temperature. A mouse anti-AFP antibody (200 ng/mL, Biomed Co.) standard was added to the first row of the plate, and serum samples were added to the other rows. The standard and the samples were titered by a factor of 1:2 with 10% FBS in PBS to obtain a final volume of 100 μL in each well, and incubated for 2 hr at room temperature. Subsequently, a sandwich ELISA was developed with horseradish peroxidase-conjugated goat anti-mouse IgG (Sigma) and substrate ABTS (2,2'-azino-bis [3-ethylbenzthiazoline-6-sulfonic acid]). The absorbance was measured at 405 nm using an ELISA plate reader. The antibody responses generated by the naked AFP DNA and pCLP7/AFP-transformed *L. casei* were quantitated based on the mouse anti-AFP standard.

The peak IgG titers of anti-AFP antibody responses were detected six weeks after administration of pCLP7/AFP-transformed *L. casei* and pcDNA/AFP. As shown in the figure attached hereto, the mice vaccinated with pCLP7/AFP-transformed *L. casei* cells had IgG titer 5 folds that of the mice vaccinated with pcDNA/AFP.

3. I hereby declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Date: April. 16. 2002

Wei-Yu Lo

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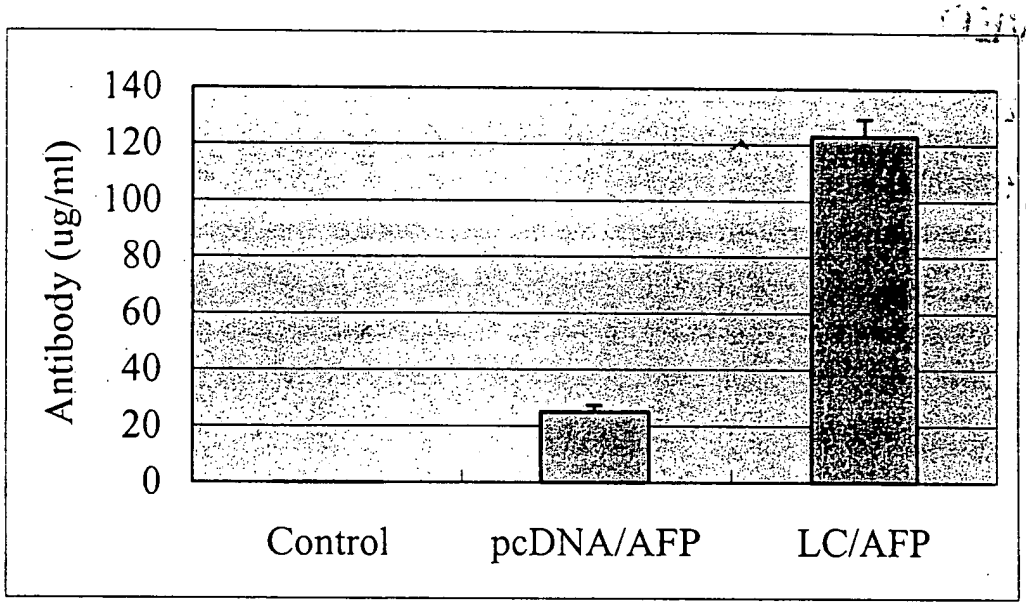
Antibody (ug/ml)
140
120
100
80
60
40
20
0
Control
pcDNA/AFP
LC/AFP